

# **Plankton Analysis by Automated Submersible Imaging Flow Cytometry: Transforming a Specialized Research Instrument into a Broadly Accessible Tool and Extending its Target Size Range**

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## **LONG TERM GOALS**

Detailed knowledge of the composition and characteristics of the particles suspended in seawater is crucial to an understanding of the biology, optics and geochemistry of the oceans. The composition and size distribution of the phytoplankton community, for example, help determine the flow of carbon and nutrients through an ecosystem and can be important indicators of how coastal environments respond to anthropogenic disturbances such as nutrient loading and pollution. Our goal is to provide researchers with instruments to continuously monitor phytoplankton community structure and investigate questions about the world's ocean ecosystems.

## **OBJECTIVES**

Flow cytometry is one of the most promising technologies for studies of the microscopic constituents of marine ecosystems (Moore et al. 2009; Sosik et al. 2009). The intent of this project is twofold: to commercialize a field-proven state-of-the-art submersible imaging flow cytometer for nano- and microplankton so that other researchers can utilize this exciting new technology, and to develop a next generation of the instrument with extended measurement range, capable of analyzing cells from pico- to microplankton.

## **APPROACH**

We will develop a prototype commercial version of Imaging FlowCytobot in a close collaboration between the WHOI developer/users and Cytopeia engineers, reproducing its functions via a series of modular components whose integration will result in a simple and robust instrument that is both reliable and easy to manufacture. The first step will involve a ground-up examination of an existing benchtop version of Imaging FlowCytobot by the Cytopeia engineering team (at WHOI). This examination will establish design goals for each functional module of the instrument (e.g., flow system, cell detector, imaging system, signal processing electronics, control system). The redesign

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process will begin with a mechanical backbone analogous to the optical breadboard now used, onto which will be designed core functional modules for cell detection and imaging, to establish a working imaging system that utilizes electronics and fluidics similar to those in the present Imaging FlowCytobot. This approach will enable us to compare performance of the commercial prototype to that of the original instrument at an early stage of development. Problems with components or integration (such as incorrect physical layout or optical components) will be corrected by consultation between WHOI researchers and Cytopeia engineers, followed by redesign and fabrication of new components. After the image quality of this core system is shown to be satisfactory, we will continue with redesign and evaluation of the other aspects of the instrument. When the upgraded benchtop unit has been satisfactorily tested in the laboratory, we will construct a pressure housing for it and conduct field tests. In collaboration with University of Washington (E. Armbrust's laboratory), we will investigate approaches to efficiently obtain both large- and small-dimension laser spots (dual beam), for simultaneous detection of pico- and microphytoplankton, using a newly-developed position sensitive detector.

## **WORK COMPLETED**

Project PIs and senior personnel met at WHOI (in October 2008) to outline the strategy for transitioning Imaging FlowCytobot to a prototype commercial product. Target goals for system improvement were identified along with methods for achieving payload, power, and cost savings. We also discussed preliminary ideas for integrating the picoplankton (position sensitive) detector into the Imaging FlowCytobot design. The WHOI partners have been investigating ways to reduce size and power requirements, and increase duration, as described below.

**Syringe pump.** The commercially-available syringe pump system we use at present takes 22W, while the syringe motor of a custom designed (by MBARI engineers) replacement uses < 1W. We found that the MBARI mechanical design is not exactly applicable to our needs (e.g., to have the syringe and distribution valve located directly above the flow cell) so we are modifying it, but we expect the power use to remain at the same low level.

**Flash Illumination.** The xenon flash lamp is not a major power consumer, but it is relatively large and expensive and its flash has a significant afterglow, so we would like to replace it by an LED. We have tried several approaches, including a more sensitive camera, the latest high power LEDs, LED arrays, and fiber optic collection of LED output (all over-driven for microsecond time periods to produce the brightest possible flash). Unfortunately, none of these attempts have produced results comparable to those of the flash lamp. We have one more approach to test; if that is not successful, we will continue to use the flash lamp, but will reduce the size of the lamp housing and fiber optic delivery system.

**Computer.** We are acquiring a new computer based on an Atom processor, which uses less than half the power of the computer we use now. If this computer proves to be compatible with the Matrox frame grabber board, it should provide a savings of ~10 W, and be faster than our present system.

**Long-duration syringe.** In response to our request, the syringe manufacturer (Kloehn, Inc.) is developing a syringe with tighter tolerances for improved cold-water performance (and longer lifetime). This will eliminate the need to manually select syringes.

Backup sheath pump. The motors in our current micro gear pumps (which we selected because of their low power requirement) have a design lifetime of ~1 yr (though some have lasted much longer). Since these are relatively expensive, we don't want to replace them for every deployment, so we have now installed 2 pumps in parallel, under computer control. This will allow us to run a motor until it fails, but then continue on the backup pump for the remainder of a deployment.

The BD Biosystems (formerly Cytocopia) partners designed and fabricated a prototype of the fixed flow cell / detector block (Fig. 1), and the WHOI partners evaluated its performance and suggested improvements. The BD engineers will visit WHOI on June 29, 2009 for hands-on testing and demonstrations to confirm the needed design changes. In the course of discussions about the optical layout, we realized that it might be possible to increase the resolution of images (by using an objective with higher numerical aperture and a flow cell that produces a thinner sample core stream); this possibility is being investigated by the WHOI partners.

## **RESULTS**

We have produced two new benchtop prototypes: one incorporating a more rigid optical structure fabricated by BD Biosystems and the other a flexible platform for easily evaluating specific components (e.g., LEDs, objectives) without need for specialized adaptors. We have produced a new prototype syringe pump and selected a new energy efficient computer. These changes combine to reduce the instrument's power requirement significantly, which will enhance its utility for non-cabled platforms. We have also identified improved syringes and implemented and tested a dual gear pump system that will allow longer deployments, increase reliability, and reduce maintenance costs.

## **IMPACT/APPLICATIONS**

### **National Security**

There is potential for this application to be useful for detecting pathogens in water supplies.

### **Economic Development**

The Imaging FlowCytobot represents a potential new product line, since it has utility for plankton ecologists studying plankton processes (including effects of pollution and climate and change), and also for water resource managers (as a means to monitor harmful algal species).

### **Quality of Life**

Species-level information is critical for such societally important problems as understanding the regulation and fate of regional harmful algal blooms. At the global scale, it is becoming increasingly evident that simple nutrient-phytoplankton-zooplankton models are inadequate for predicting effects of environmental change and that biogeochemical functional groups such as nitrogen fixers, silicifiers, and calcifiers need be resolved. We presently lack observational capabilities to provide data for building and evaluating models, as well as for developing new approaches such as satellite-based remote sensing approaches to monitor functional group distributions. Widespread availability of instruments such as Imaging FlowCytobot will be an important step to overcoming present observational limitations.

### **Science Education and Communication**

The images of individual plankton cells provided by these instruments, remotely and in near-real time, should contribute effective components of educational programs about the oceans, both in science curricula and for the general public.

### **TRANSITIONS**

#### **Quality of Life**

A prototype Imaging FlowCytobot has already provided early warning of a toxic dinoflagellate bloom in the Gulf of Mexico (the first toxic *Dinophysis* bloom observed in Texas waters), allowing timely closure of shellfisheries that prevented human illnesses.

### **Science Education and Communication**

Images from a prototype Imaging FlowCytobot have been circulated to plankton experts via the Internet, allowing species identification and better interpretation of potential processes behind bloom dynamics.

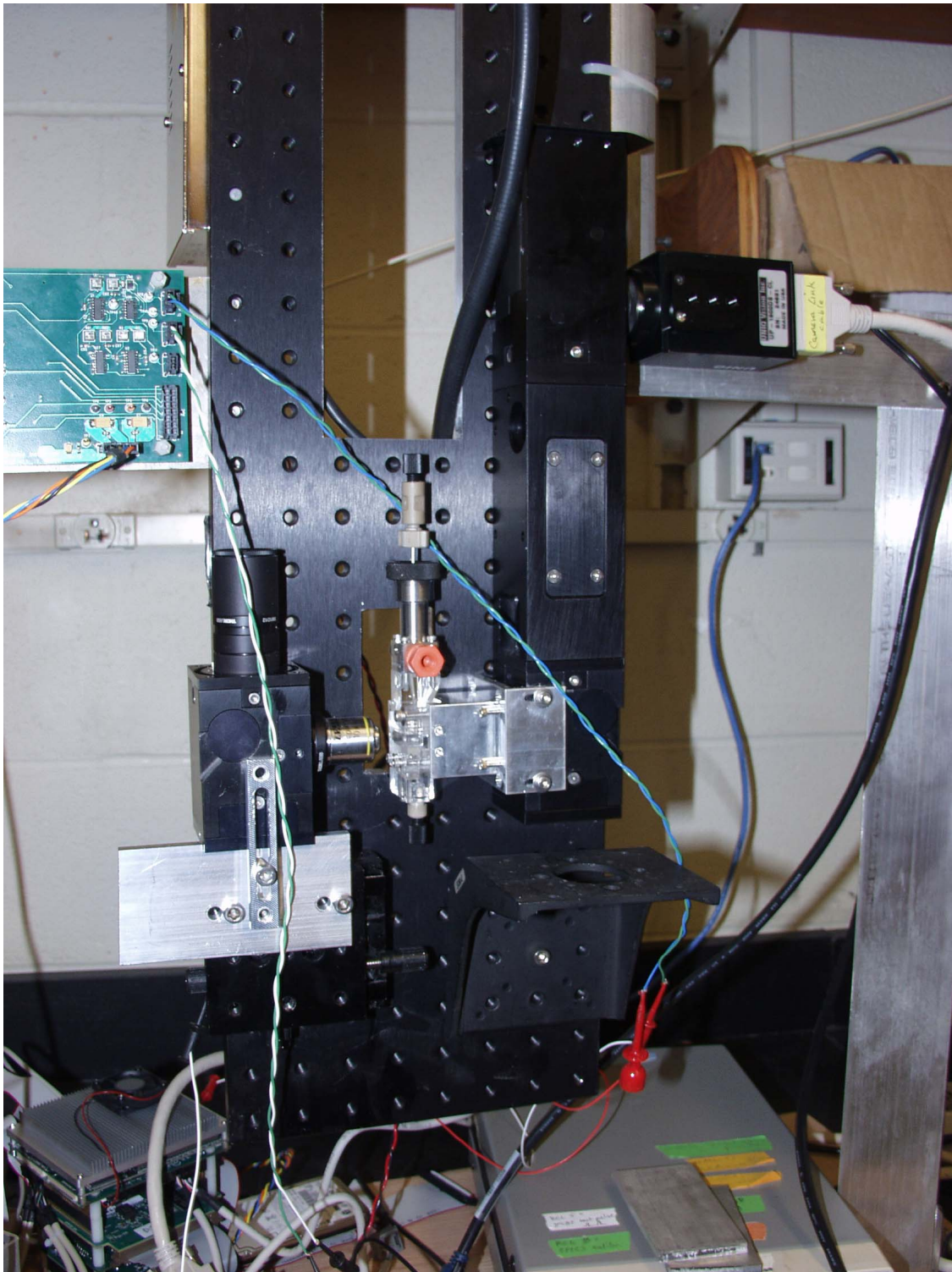
### **RELATED PROJECTS**

This project builds on previous projects in the Olson and Sosik laboratories. See <http://www.whoi.edu/sites/hsosik/> for more details.

### **PUBLICATIONS**

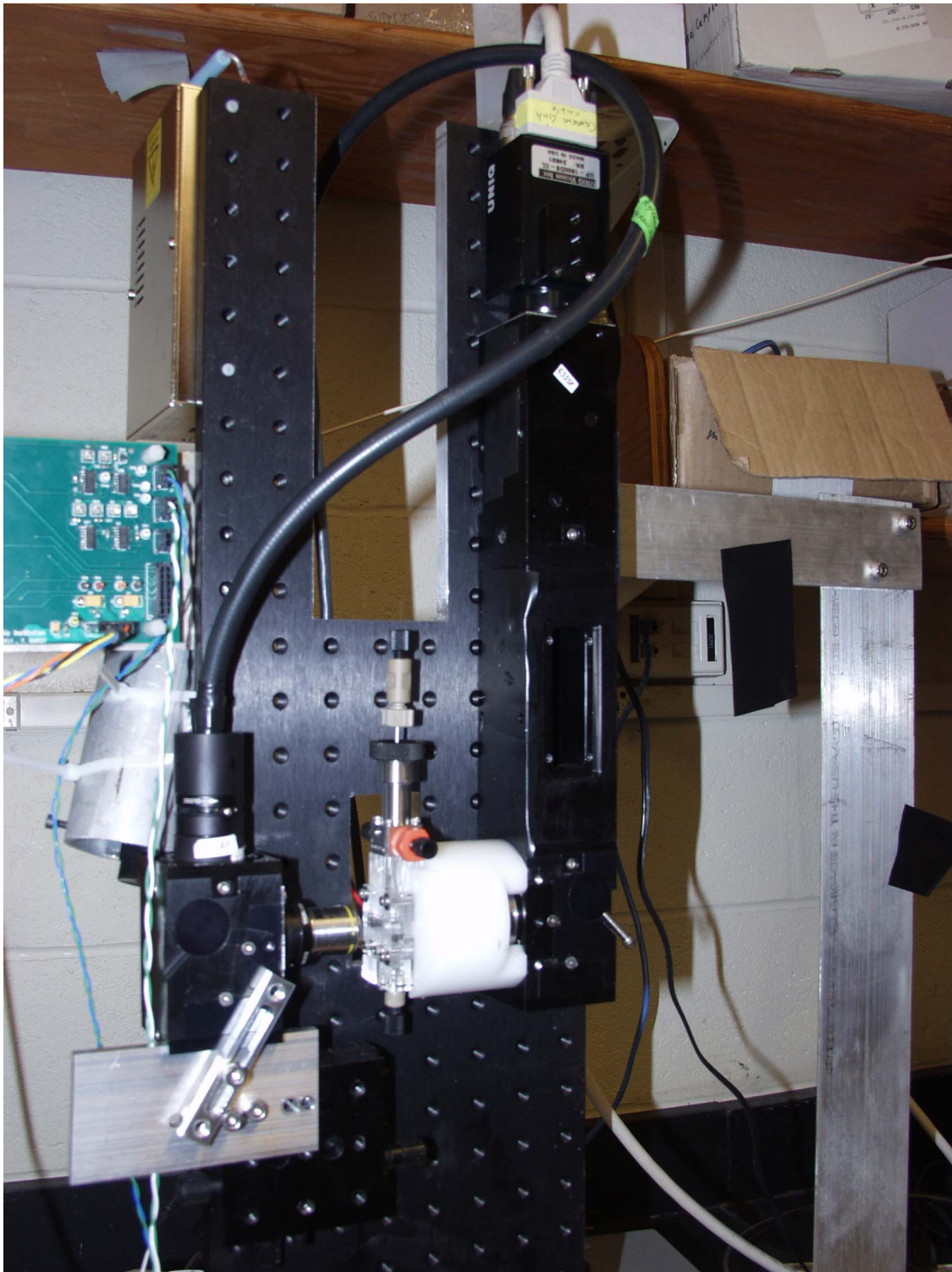
Moore, C., A. Barnard, P. Fietzek, M. R. Lewis, H. M. Sosik, S. White, and O. Zielinski. 2009. Optical tools for ocean monitoring and research. Ocean Science in revision; <http://www.ocean-sci-discuss.net/5/659/2008/osd-2005-2659-2008.html>.

Sosik, H. M., R. J. Olson, and E. V. Armbrust. 2009. Flow cytometry in plankton research, p. in press. In D. J. Suggett, O. Prasil and M. A. Borowitzka [eds.], Chlorophyll a fluorescence in aquatic sciences: methods and applications. Springer. in press.

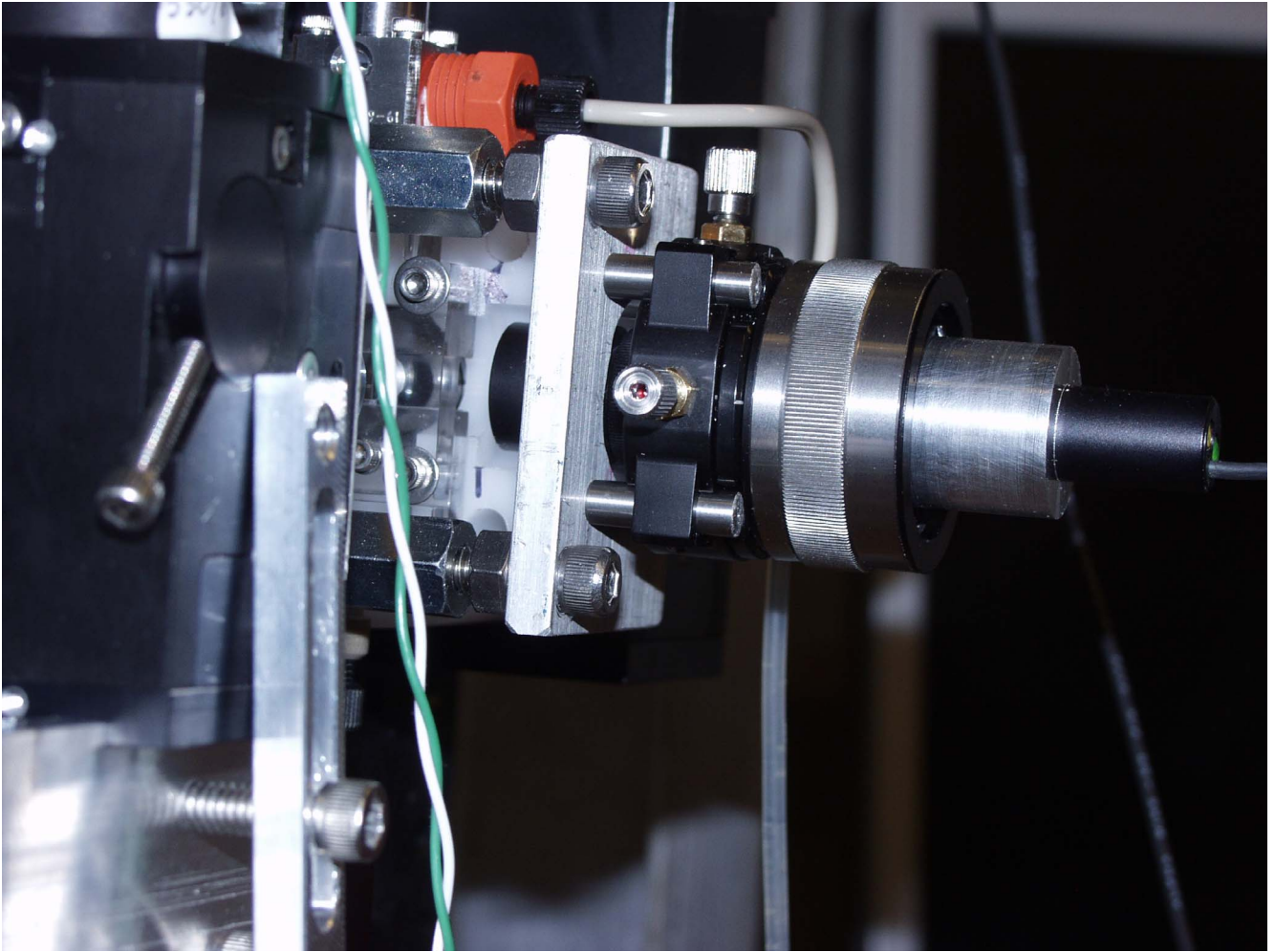


*Fig. 1. Prototype Flow cell/detector block (version 1) from BD partners.*



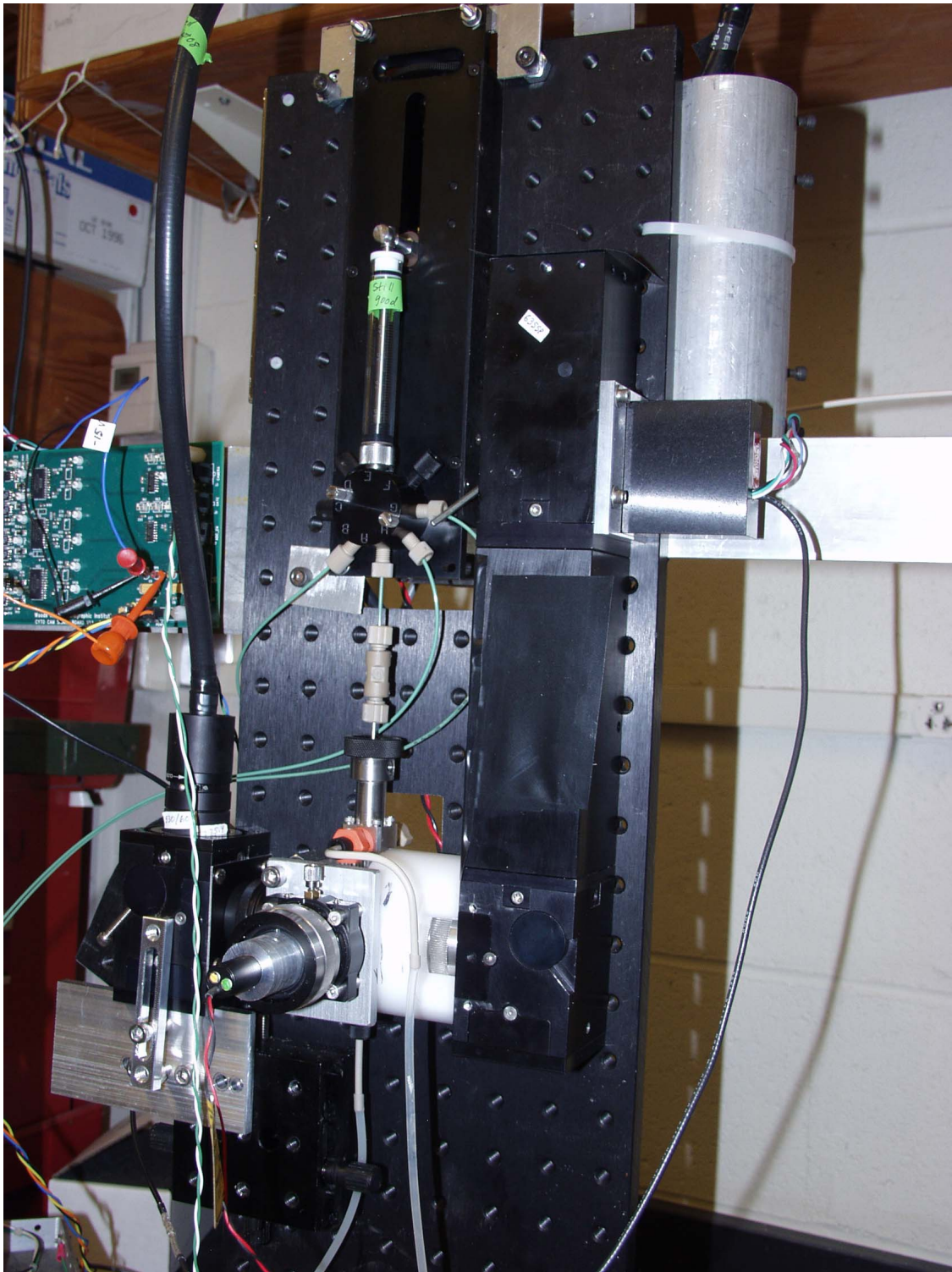


*Fig. 2. Flow cell / detector block (version 2), WHOI modifications (fine-focus objective mount, camera in line).*



*Fig. 3. Laser mount closeup.*





*Fig. 4. Working prototype (flash lamp images triggered by PMT signals from particles flowing through laser beam) [camera removed here].*